

WHAT IS CLAIMED:

Claims

1. An isolated recombinant anti-CD3 antibody, or antigen-binding fragment thereof, comprising:
 - a) a heavy chain comprising a heavy chain complementarity determining region (HCDR) 1 comprising SEQ ID NO: 662; a HCDR2 comprising SEQ ID NO: 663; and a HCDR3 comprising SEQ ID NO: 664 and a light chain comprising a light chain complementarity determining region (LCDR) 1 comprising SEQ ID NO: 671, a LCDR2 comprising SEQ ID NO: 673, and a LCDR3 comprising SEQ ID NO: 690;
 - b) a heavy chain variable region comprising SEQ ID NO: 652 and a light chain variable region comprising SEQ ID NO: 661;
 - c) a heavy chain comprising SEQ ID NO: 640 and a light chain comprising SEQ ID NO: 676;
 - d) a heavy chain variable region comprising SEQ ID NO: 657 and a light chain variable region comprising SEQ ID NO: 678; or
 - e) a heavy chain comprising SEQ ID NO: 675 and a light chain comprising SEQ ID NO: 677.
2. ~~An~~The isolated recombinant anti-CD3 antibody or antigen-binding fragment thereof, of claim 1, wherein the antibody or antigen-binding fragment specifically binds *Macaca fascicularis* or human CD3d, or CD3e, or CD3e and CD3d with a binding affinity of about 300 nM or less.
3. The isolated recombinant anti-CD3 antibody or antigen-binding fragment thereof of claim 2, wherein the binding affinity is about 100 nM or less.
4. The isolated recombinant anti-CD3 antibody or antigen-binding fragment thereof of claim 2 ~~or 3~~, wherein the binding affinity is measured by flow cytometry or by ~~Proteon~~ProteOn surface plasmon resonance assay ProteOn XPR36 system at +25°C.
5. The isolated recombinant anti-CD3 antibody or antigen-binding fragment thereof of ~~any one of the previous claims~~claim 2, wherein the antibody or antigen-binding fragment has one, two, three, or four of the following properties:
 - a) binds human and *Macaca fascicularis* CD3+ T lymphocytes with a calculated EC50 of 300 nM or less and binds *Macaca fascicularis* CD3-expressing HEK cells with a calculated EC50 of 300 nM or less, wherein the difference in calculated EC50 between binding CD3+ T lymphocytes and binding *Macaca fascicularis* CD3-expressing HEK cells is less than 5-fold, and wherein the calculated EC50 is measured in a whole cell binding assay at 0 °C using flow cytometry;

- b) binds recombinant CD3d from human (SEQ ID NO:691), or binds recombinant CD3e from human (SEQ ID NO:636), or binds recombinant CD3d from *Macaca fascicularis* (SEQ ID NO:692), or binds recombinant CD3e from *Macaca fascicularis* (SEQ ID NO:693) with an equilibrium dissociation constant (K_D) of 300 nM or less, wherein the K_D is measured using Proteon surface plasmon resonance assay ProteOn XPR36 system at +25°C;
 - c) binds residues 1-6 of CD3e as determined by X-ray crystallography; or
 - d) activates T cells or induces CD69 expression to a similar degree as cOKT3 or SP34-2 as determined by fluorescence-activated cell sorting assay.
6. The antibody or antigen-binding fragment thereof of ~~any one of the previous claims~~claim 2 comprising at least one substitution in an antibody constant domain, the at least one substitution comprising:
 - a) heavy chain substitutions K409R, F405L, or F405L and R409K;
 - b) heavy chain substitutions S228P, F234A, and L235A;
 - c) heavy chain substitutions L234A, G237A, P238S, H268A, A330S and P331S, wherein the antibody is an IgG1 isotype; or
 - d) heavy chain substitution S228P, wherein the antibody is an IgG4 isotype;
 wherein residue numbering is according to the EU Index.
 7. The antibody or antigen-binding fragment thereof of ~~any one of the previous claims~~claim 2, comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of SEQ ID NOs:662, 663, 664, 671, 673, and 690, respectively.
 8. The antibody or antigen-binding fragment thereof of ~~any one of the previous claims~~claim 2, comprising at the heavy chain variable region (VH) and at the light chain variable region (VL) of SEQ ID NOs: 652 and 661, respectively.
 9. The antibody or antigen-binding fragment thereof of ~~any one of the previous claims~~claim 2, comprising at the heavy chain sequence (HC) and at the light chain sequence (LC) of SEQ ID NOs: 640 and 676, respectively.
 10. The antibody or antigen-binding fragment thereof of ~~any one of claims 1-5~~claim 2, comprising a VH and a VL of SEQ ID NOs:657 and 678, respectively.
 11. The antibody or antigen-binding fragment thereof of ~~any one of claims 1-5~~claim 2, comprising a HC and a LC of SEQ ID NOs:675 and 677, respectively.

12. An antibody or antigen-binding fragment thereof, comprising ~~thea~~ HCDR1, ~~thea~~ HCDR2, ~~thea~~ HCDR3, ~~thea~~ LCDR1, ~~thea~~ LCDR2, and ~~thea~~ LCDR3 of SEQ ID NOs: 662, 663, 664, 671, 673, and 690, respectively.
13. An antibody or antigen-binding fragment thereof, comprising a VH and a VL of SEQ ID NOs:652 and 661, respectively.
14. An antibody or antigen-binding fragment thereof, comprising a HC and a LC of SEQ ID NOs:640 and 676, respectively.
15. An antibody or antigen-binding fragment thereof, comprising a VH and a VL of SEQ ID NOs:657 and 678, respectively.
16. An antibody or antigen-binding fragment thereof, comprising a HC and a LC of SEQ ID NOs:675 and 677, respectively.
17. ~~The antibody or antigen-binding fragment thereof of any one of the previous claims~~claim 2, wherein the antibody is human or humanized.
18. The antibody of claim 17, wherein the antibody is an IgG4 or IgG1 isotype.
19. The antibody of claim 18, comprising one, two, three, four, five, six, seven, eight, nine or ten substitutions in the antibody Fc.
20. -An antibody or antigen-binding fragment thereof, comprising a HC of SEQ ID NO:~~639~~640 and a LC of SEQ ID NOs:646, comprising at least one substitution comprising:
 - a) D43G, L49M, L50I, S62N, Q85E light chain substitutions;
 - b) D43G, V48L, L49M, L50I, S62N, Q85E, H89Y light chain substitutions;
 - c) R10G, R13K, V73I, R70K, T83S, L96V heavy chain substitutions;
 - d) any one of light chain substitutions D43G, V48L, L49M, L50I, S62N, Q85E, or H89Y; or
 - e) any one of heavy chain substitutions R10G, R13K, V73I, R79K, T83S, or L96V,
 wherein residue numbering is according to the EU Index.
21. The antibody of ~~any one of the previous claims~~claim 2, wherein the antibody is bispecific or multispecific.
22. A bispecific antibody comprising a first domain that specifically binds CD3 and a second domain that specifically binds a second antigen, wherein the first domain comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of SEQ ID NOs:662, 663, 664, 671, 673, and 690, respectively.

23. The bispecific antibody of claim 22 wherein the first domain and second domain are an IgG4 isotype, and wherein the first or the second domain comprises S228P, F234A, L235A, F405L and R409K heavy chain substitutions and the other domain of the first or the second domain comprises S228P, F234A and L235A heavy chain substitution, wherein residue numbering is according to the EU Index.
24. The bispecific antibody of claim 22, wherein the first and/or the second domain comprises at least one substitution in a CH3 constant domain comprising a F405L, or F405L and R409K substitution, wherein residue numbering is according to the EU Index.
25. The bispecific antibody of claim 22, wherein one of the first or the second domains comprises a F405L heavy chain substitution and the other of the first or second domains comprises a K409R heavy chain substitution, wherein residue numbering is according to the EU Index.
26. The bispecific antibody of claim 22, wherein the first domain and the second domain are an IgG4 isotype, wherein one of the first or the second domains comprises a S228P heavy chain substitution and the other of the first or the second domains comprises S228P, F405L and R409K heavy chain substitutions, wherein residue numbering is according to the EU Index.
27. The bispecific antibody of claim 22, wherein the first domain comprises the VH and the VL of SEQ ID NOs: 652 and 661, respectively.
28. The bispecific antibody of claim 22, wherein the first domain comprises the HC and the LC of SEQ ID NOs: 640 and 676, respectively.
29. The bispecific antibody of claim 22, wherein the first domain comprises the VH and the VL of SEQ ID NOs: 657 and 678, respectively.
30. The bispecific antibody of claim 22, wherein the first domain comprises the HC and the LC of SEQ ID NOs: 675 and 677, respectively.
31. The bispecific antibody of claim 22, wherein the second antigen is a cell surface antigen that is expressed on a target cell other than an immune effector cell.
32. The bispecific antibody of claim 31, wherein the cell surface antigen is a tumor associated antigen.
33. The bispecific antibody of ~~any one of claims 24-34~~ claim 22, wherein the second antigen is CD33, IL1RAP, PSMA or TMEFF2.

34. A pharmaceutical composition comprising the antibody of ~~any one of the previous claims~~claim 1 and a pharmaceutically acceptable carrier.
35. A polynucleotide encoding the antibody of ~~any one of the claims 1-34~~claim 2.
36. A vector comprising the polynucleotide of claim 35.
37. A host cell comprising the vector of claim 36.
38. A method of producing the antibody of ~~any one of claims 1-34~~claim 2, comprising culturing the host cell of claim 37 in conditions that the antibody is expressed, and recovering the antibody produced by the host cell.
39. ~~A method of treating~~Use of the isolated antibody of claim 2 in the manufacture of a medicament for the treatment of cancer in a subject, comprising administering a therapeutically, wherein an effective amount of the isolated antibody of any one of claims 1-34 is to be administered to a subject in need thereof for a time sufficient to treat the cancer.
40. The ~~method~~use of claim 39, wherein the cancer is a solid tumor or a hematological malignancy.
41. The ~~method~~use of claim 40, wherein the solid tumor is a prostate cancer, a colorectal cancer, a gastric cancer, a clear cell renal carcinoma, a bladder cancer, a lung cancer, a squamous cell carcinoma, a glioma, a breast cancer, a kidney cancer, a neovascular disorder, a clear cell renal carcinoma (CCRCC), a pancreatic cancer, a renal cancer, a urothelial cancer or an adenocarcinoma to the liver.
42. The ~~method~~use of claim 41, wherein the prostate cancer is a refractory prostate cancer, a prostatic intraepithelial neoplasia, an androgen independent prostate cancer, or a malignant prostate cancer.
43. The ~~method~~use of claim 40, wherein the hematological malignancy is acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic myeloid leukemia (CML) or blastic plasmacytoid dendritic cell neoplasm (DPDCN).
44. The ~~method~~use of ~~any one of claims 38-43~~claim 39, wherein the antibody is to be administered in combination with a second therapeutic agent.
45. The isolated antibody of any one claim 2 for use in the treatment of cancer, wherein an effective amount of the antibody is to be administered to a subject in need thereof for a time sufficient to treat the cancer.

45. 46. The antibody of claim 1-34 for use in therapy.